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Indirect measurement of lymphatic absorption in CAPD patients is not influenced by trapping

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Indirect measurement of lymphatic absorption in CAPD patients is not influenced by trapping. The disappearance rate of intraperitoneally administered macromolecules is often used to calculate lymphatic absorption during CAPD. The possible contribution of local accumulation (trapping) of such solutes in the tissues surrounding the peritoneal cavity, leading to overestimation of lymphatic flow, was investigated in eight CAPD patients. They were studied twice during a four hour dwell, glucose 1.36%, to which polydisperse neutral dextran 70 1 g/liter had been added for measurement of lymphatic flow. After the test on day 1 dextran 130 mg/kg was given intravenously and also dextran 1 g/liter was added to every following dialysis bag until the second test on day 3. This was done to saturate the tissues surrounding the peritoneal cavity and thereby to create a steady state condition. In one patient the dextran administration was continued until a third study was done on day 5. Dextran in serum during day 3 was 1.3 ± 0.5 g/liter (mean ± SD). No difference in peritoneal clearance of dextran was found between day 1 and day 3 (1.11 ± 0.56 versus 0.97 ± 0.41 ml/min). Also no difference was found between day 1 (0.32), day 3 (0.62), and day 5 (0.42 ml/min). Trapping would have influenced the first but not the second test, as the second time all tissues were saturated with dextran. As the dextran absorption rate remained the same, this indicates that trapping is of no importance and that lymphatic absorption can be measured by the disappearance of a macromolecular marker.

Fluid can be absorbed from the peritoneal cavity either by transport across the walls of the peritoneal capillaries (transcapillary absorption) or by lymphatic absorption. In experimental and human studies, the amount of fluid removed by lymphatic transport has been quantitated with the use of macromolecules added to the intraperitoneal fluid [1–4]. When lymphatic transport is calculated using data on macromolecular transport, it is assumed that these high molecular weight substances are transported into the lymphatics without molecular sieving.

The transport of macromolecules from blood to dialysate through the peritoneal capillaries is size-selectively restricted. This limitation is caused either by restricted diffusion [5] or by filtration through pores [6]. The passage of macromolecules from the peritoneal cavity to blood is considered to be mediated mainly by lymphatic absorption, and possibly also to a limited extent by restricted diffusion. Evidence that macromolecules are removed by lymphatic absorption is given by the finding that these substances disappear from the peritoneal cavity independently of their molecular weight [7] and also appear in blood at the same time after intraperitoneal administration, independently of their molecular weight [8–10]. Convective transcapillary transport from the abdominal cavity to the circulation during a four hour dwell can be neglected for these high molecular weight solutes [6].

However, controversy exists whether lymphatic absorption should be calculated using the appearance in blood or the disappearance from the peritoneal cavity of intraperitoneally administered macromolecules [11–13]. Calculating the lymphatic absorption as the disappearance rate of a macromolecule yields a considerably higher lymphatic flow rate independent of the solute used, than calculation of the appearance rate in plasma. In rats, intraperitoneally administered macromolecules disappear from the peritoneal cavity at a constant rate [2]. After a four hour dwell in CAPD patients 15 to 20% of the administered quantity is lost [14]. From the disappearance of these solutes a clearance of macromolecules of about 1.5 ml/min can be calculated [3, 15, 16]. However, the appearance in the systemic circulation is only 11 to 20% of the amount lost after a dwell time of four to eight hours, giving a estimated lymph flow of about 0.20 ml/min [4, 17]. Only in anesthetized sheep no discrepancy was found between the appearance and the disappearance of radiolabeled albumin. In that model two out of three lymphatic pathways from the peritoneal cavity could be cannulated and were found to be responsible for over 40% of the amount lost from the peritoneal cavity [18].

In theory, the low appearance rate of a marker into the circulation could be explained by underestimation of the distribution volume of the marker, or neglecting losses due to residual renal function. When the recovery of a marker is correctly estimated, there are two possible explanations for the differences found between the two methods of calculating lymphatic absorption. The discrepancy between the two methods is probably caused by the absence of steady state conditions: the macromolecular marker has been administered during only one dwell in the previous studies. The first explanation is that the marker disappears in the tissues surrounding the peritoneal cavity, followed by local accumulation (trapping). Then the disappearance rate would overestimate lymphatic absorption. Such trapping, especially in the anterior abdominal

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wall has been reported in rats by Flessner et al using radiolabelled albumin [19, 20]. When this mechanism would be important in CAPD patients, the disappearance rate of intraperitoneally administered macromolecules should decrease after saturation of these tissues, as can be achieved after repeated administration. The second explanation for the large difference between the peritoneal disappearance rate of macromolecules and the appearance rate in plasma could simply be the slow passage of these substances through the peritoneal interstitial tissues before entering the lymphatic vessels or through the lymphatic system itself, resulting in a delayed appearance in the circulation. In that case the disappearance rate of intraperitoneally given macromolecules should not be influenced by repeated administration. If so, it would imply that lymphatic absorption can be measured in a reliable way from the disappearance rate of the macromolecular tracer during a single dialysis dwell.

To elucidate the possible role of trapping during non-steady state conditions we studied the disappearance of dextran 70 from the peritoneal cavity twice in eight CAPD patients: the first time in a non-steady state condition using a single gift of dextran 70 and the second time during steady state conditions obtained after a single intravenous loading dose of dextran 70 followed by continuous administration of dextran 70 in the dialysate for two days.

Methods

Patients

Five male and three female CAPD patients were studied twice with an interval of 48 hours (study 1 on day 1 and study 2 on day 3). One patient was also studied a third time on day 5. Median age was 42 years (range 27 to 65) and median duration of CAPD treatment two months (range 1 to 24). The cause of renal failure in these patients was chronic pyelonephritis in three, and chronic interstitial nephritis, chronic glomerulonephritis, lupus nephritis, diabetic nephropathy and polycystic disease in one. All patients were treated with four exchanges per day, five patients using 1.5 liters and three using 2 liters dialysate. None of the patients had peritonitis during or in the three weeks before the study.

In addition, six other CAPD patients (5 males, 1 female) were studied once to investigate whether the disappearance of the marker used was linear in time (study 3). Median age of these patients was 69 years (range 56 to 77) and median duration on CAPD 14 months (range 8 to 39).

Informed consent was obtained from all patients after explanation of the purpose of the study. The protocol was approved by the medical ethics committee of the University Hospital of Amsterdam.

Procedure

The studies were done with commercially available prewarmed dialysate, glucose concentration 1.36% (Dianeal®, Baxter B.V., Utrecht, The Netherlands). All exchanges were performed with the dialysate volume the patients were used to. The patients were examined twice in the same semi-recumbent position. Prior to the study the abdominal cavity was rinsed twice with dialysate that was drained immediately after inflow. On day 1 20 ml of dextran 1 (Promiten®, NPBI, Emmercompascuum, The Netherlands) was given intravenously before the test. This was done to minimize the possible occurrence of anaphylaxis to dextran [21]. Then a fresh dialysate bag (test bag) containing inulin (Inutest®, Laevosan-Gesellschaft, Linz/Donau, Austria) 2.5 g/liter and dextran 70 1 g/liter (6% dextran in glucose 5%, Macrodex®, NPBI, Emmercompascuum, The Netherlands) was instilled for a dwell time of exactly four hours. After drainage of the test bag the abdominal cavity was rinsed again with glucose 1.36%. After the test the patients resumed CAPD on their normal schedule.

Starting with the test bag on day 1 dextran 70 1 g/liter was added to each dialysate bag up to and including the test bag on day 3. In one patient dextran was administered intraperitoneally until the third test on day 5. After drainage of the test bag on day one, dextran 70, 130 mg per kg body wt, was given intravenously.

Dialysate samples of the test bag were taken before inflow, after 10, 20, 30, 60, 120 and 180 minutes and after drainage. Samples before inflow and after drainage were also obtained from the rinsing bags. Blood was taken during inflow and four hours later during outflow of the test bag.

In the other patient group studied once (study 3), the test procedure was similar to the test on day 1. In contrast to the test on day 1, dialysate samples between start and end of the test were always taken after complete drainage of the peritoneal cavity, followed again by immediate inflow, at 60, 120, and 180 minutes. This study was completed after rinsing the peritoneal cavity.

Methods

For the measurement of total dextran concentration in the dialysate 1.25 ml of the sample was mixed with 0.25 ml trichloroacetic acid (1 g/ml) to precipitate the proteins. It was then left to incubate at 45°C for one hour to hydrolyze the inulin. After centrifugation at 3000 rpm for 10 minutes 1.0 ml of the supernatant was brought on a PD-10 column (Pharmacia LKB, Uppsala, Sweden) to obtain further purification. The first milliliter of eluens (phosphate buffered saline, 0.2% sodium azide) that came off the column was discarded, but the following 3.5 ml were collected and were clean enough for adequate dextran determination. This was done by high performance liquid chromatography (HPLC) using a Bio Gel TSK-XL guard column (40 × 6 mm) from BioRad Chemical Division (Richmond, Virginia, USA) to separate dextran from the matrix. The column was incorporated in an automated HPLC system containing one HPLC spectroflow 400 pump and a spectroflow 450 controller, both from ABI Kratos Analytical (Ramsey, New Jersey, USA), a PROMIS sample processor from Spark Holland (Emmen, The Netherlands), an ERMA 7510 refraction index detector from ERMA (Tokyo, Japan) and an SP 4290 integrator (Spectra Physics, Eindhoven, The Netherlands). The latter was used in its peak height mode. As a consequence of the use of the short column as the separating unit, the chromatographic analysis time could be reduced to only seven minutes for one sample. The coefficient of variation of the method in the concentration range of 200 mg/liter to 5,000 mg/liter was 2.2%. To further improve the accuracy all samples were measured in duplicate.

Urea was measured by use of the DAM-TSC Technicon SD4-001 DK 7 method, creatinine by the modified Jaffé method

and glucose by the glucose oxidase-peroxidase method, all determined by autoanalyzers (Technicon SMA and SMA-II). The creatinine values obtained in dialysate were corrected using creatinine measurements in blanks, because of interference with the high glucose concentration of the dialysate. L-Lactate was measured by a lactate dehydrogenase method (fully enzymated method, no. 149993, Boehringer, Mannheim, Germany). Inulin was measured by a modification of Walser's method with the color reagent diphenylamine [22], selected because interference with the high glucose concentration of the dialysate is absent with this method. The coefficient of variation of the inulin determinations was 4%. To further improve the accuracy all samples were measured in triplicate. Albumin and IgG were measured by immunoturbidimetry using commercial antisera (Dakopatts, Gotrupp, Denmark). The coefficient of variation of the protein determinations was 2%. Appropriate control sera were used in each series of measurements.

Calculations

Each experiment was analyzed individually for the determination of the dextran absorption rate (DAR), peritoneal solute transport kinetics and net fluid removal. The calculations of DAR were done using the following equation:

$$\text{DAR (ml/min)} = \frac{\text{Dex}_{in} - \text{Dex}_{out}}{\text{D}_{geom}t} \quad (1)$$

Parameters used in this equation are:

Dex_{in} (in mg). The amount of dextran present at the start of the test, calculated on day 1 as the amount of dextran instilled. On day 3 and 5 the residual volume already containing dextran and therefore Dex_{in} was calculated as the sum of the amount of dextran instilled and the amount of dextran present in the residual volume before inflow.

Dex_{out} (in mg). The amount of dextran recovered after drainage, in the residual volume after drainage and in the samples obtained during the four hour test.

D_{geom} (in mg/ml). Geometric mean of the initial (D_0) and final dextran concentration (D_t) in the dialysate. D_0 is calculated as Dex_{in} of either day 1 or day 3/5, divided by the sum of the instilled volume and the residual volume before inflow.

t. Time period of the dwell (in minutes). In this approach it is assumed that DAR is constant during the dialysate dwell time of the test, as has also been found for the macromolecule albumin in rats [2]. Furthermore, it is assumed that the disappearance of dextran from the peritoneal cavity is entirely due to lymphatic absorption. It is implied that in the study on day 1 the DAR was overestimated caused by the diffusive solute transport through the peritoneal membrane from the peritoneal cavity to the blood. In the study on days 3 and 5 the DAR was either overestimated or underestimated depending on the concentration gradient of dextran over the peritoneal membrane. Equation 1 was corrected for this diffusive transport as follows:

$$\text{Day 1: DAR (ml/min)} = \frac{\text{Dex}_{in} - \text{Dex}_{out} - \text{D}_{geom}\text{C}_{dex}}{\text{D}_{geom}t} \quad (2)$$

$$\text{Day 3: DAR (ml/min)} = \frac{\text{Dex}_{in} - \text{Dex}_{out} - (\text{D}_{geom} - \text{P})\text{C}_{dex}}{\text{D}_{geom}t} \quad (3)$$

in which C_{dex} is the transcapillary clearance of dextran (in ml/min). Assuming symmetry for the diffusional characteristics of the peritoneal membrane (that is, dialysate to blood or blood to dialysate), C_{dex} was assumed to be 0.05 ml/min. This represents the mean clearance of dextran 70 from blood to dialysate, as was calculated for the dextran fraction of 50 Å [5], because this radius represents the fraction with the highest concentration [7]. The data that are given are corrected for transcapillary transport of dextran unless stated otherwise. The standard deviation of the measured DAR, is independent of the size of the DAR in the range of 0 to 3.0 ml/min, and was calculated to be 0.25 ml/min [23].

Lymphatic absorption was assumed to be equal to the dextran absorption. The cumulative transcapillary ultrafiltration (TCUF) at time t, that is, the change of the theoretical intraperitoneal volume in the absence of lymphatic absorption, was calculated as the amount of dextran instilled, divided by the dextran concentration at time t, minus the intraperitoneal volume at the start of the test. The difference between TCUF and lymphatic absorption was considered to represent the change of the in situ intraperitoneal volume (ΔIPV). $TCUF_1$, the transcapillary ultrafiltration from 0 to 1 minute, was calculated by the Lineweaver-Burke plot [15, 16]. The curves in Figures 2 and 3 of TCUF and ΔIPV were calculated from data on the various time points using the calculated TCUF by the method of the Lineweaver-Burke plot.

The residual volume before inflow of the test bag (RV1) was calculated using equation 4, in which V_{in} is the volume of the test bag before inflow, $C_{i,in}$ is the inulin concentration in the test bag and $C_{i,t}$ is the inulin concentration of the dialysate 10 minutes after inflow of the test bag.

The residual volume after drainage of the test bag (RV2) was calculated using equation 5, in which Vr_{in} is the volume of the rinsing bag after the test, $Cr_{i,out}$ is

$$\text{RV1 (ml)} = \frac{\text{V}_{in}\text{C}_{i,in}}{\text{C}_{i,t}} - \text{V}_{in} \quad (4)$$

the inulin concentration of the rinsing bag after drainage, and $C_{i,out}$ is the inulin concentration of the test bag after drainage.

$$\text{RV2 (ml)} = \frac{\text{Vr}_{in}\text{Cr}_{i,out}}{\text{C}_{i,out} - \text{Cr}_{i,out)}} \quad (5)$$

Although the residual volumes measured with inulin were used in calculating DAR, they were also measured using dextran before inflow of the test bag at day 1.

During study 3 the percentage recovery of dextran was calculated as Dex_{out} at the various time points the peritoneal cavity was completely drained, divided by Dex_{in} at the start of the test and multiplied by 100, assuming that the residual volume was constant during the test. This residual volume was calculated as RV2.

Mass transfer area coefficients (MTC) for urea (mol wt 60), lactate (mol wt 90), creatinine (mol wt 113), glucose (mol wt 180) and inulin (mol wt 5,500) were calculated using a mathematical model assuming first order kinetics [24–26]. Protein clearances for albumin (mol wt 69,000) and IgG (mol wt 150,000) were calculated as the amount of protein drained, divided by the product of the mean of the determinations of each protein in serum and the dwell time. Net fluid removal was

Table 1. Theoretically calculated effect of two days of i.p. administration of dextran 70 on the dextran concentration within the peritoneal interstitium and on the dextran disappearance rate into this interstitial space

Interstitial volume *liters*	Saturation of interstitium %	Clearance day 1	Clearance day 3	Difference day 1 and 3
		ml/min		
0.35	99.7	0.3 ± 0.7	0.3 ± 0	0.7
0.5	98.2	0.3 ± 0.7	0.3 ± 0.01	0.699
1	86.7	0.3 ± 0.7	0.3 ± 0.09	0.691
2	63.5	0.3 ± 0.7	0.3 ± 0.25	0.45

Calculations are based on a four hour dwell, using different interstitial volumes. At day 1 a dextran clearance of 1 ml/min is used, consisting of an assumed lymph flow of 0.3 ml/min and a dextran disappearance rate to the interstitium of 0.7 ml/min. On day 3 the dextran clearance is the sum of lymph flow and the calculated dextran disappearance rate.

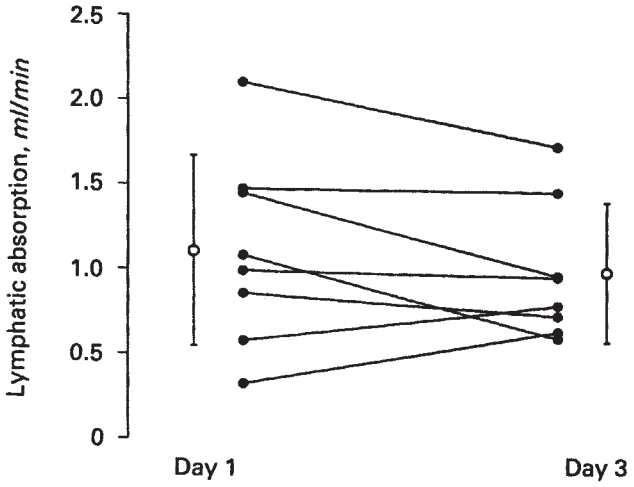

Fig. 1. *Lymphatic absorption rate (ml/min) in eight stable CAPD patients determined by the disappearance rate of dextran 70 from the peritoneal cavity before (day 1) and after (day 3) intravenous administration of dextran 70 (after correction for diffusive transport across the peritoneal capillaries).* Also mean values (○) and SD are given.

calculated by the sum of the difference in weight of the test bag before inflow and after drainage and the volume of the samples obtained during the test.

The results are given as mean values ± 1 standard deviation. Linear regression analysis was calculated by the method of least squares. Whether dextran disappeared linear in time during study 3 was tested using analysis of variance for repeated measurements [27]. Differences between the two tests were analyzed using the Wilcoxon matched-pairs test. A P value below 0.05 (two-tailed) was considered to indicate a significant difference.

Theoretical considerations on the volume of the peritoneal interstitium and the effect of saturation on the disappearance of dextran

The peritoneal interstitial volume can be calculated as the product of its peritoneal surface area and thickness. Two studies are available in which the peritoneal surface area was measured in six and eight adults. In these studies a mean peritoneal surface area of 1.04 m^2 [28] to 0.78 m^2 [29] was found. To the best of our knowledge, no data are available regarding an average depth of the peritoneum. In the mesentery the average thickness from mesothelium to mesothelium is 30 μm, with exceptions till 110 μm [30]. The parietal peritoneum is loosely attached to the abdominal wall and easily stripped [31], so the thickness is estimated to be 2 mm. The visceral peritoneum is more dense and firmly attached to the underlying tissues [31]. When looking at cross sections of the gut, it seems that the thickness of the serosa varies from 50 to 250 μm [32]. Using the average contribution of the various parts of the peritoneum to the total surface area [28, 29], the following peritoneal interstitial space can be calculated: mesentery and omentum 30% of 1 m^2 × 50 μm = 0.015 liters, viscera 60% of 1 m^2 × 150 μm = 0.09 liters and parietal peritoneum 10% of 1 m^2 × 2 mm = 0.2 liters, giving a total peritoneal interstitial volume of about 0.35 liters.

When the disappearance rate of dextran is 1.0 ml/min and it is assumed that lymph flow is constant at a rate of 0.3 ml, the transport rate of dextran to the interstitium is 0.7 ml/min. Then the dextran concentration in the interstitium after two days of saturation can be calculated using a simple kinetic model (equation 6) [25]:

$$\text{MTC (ml/min)} = \frac{V}{t} \ln\left(\frac{D_0 - I_0}{D_t - I_t}\right) \quad (6)$$

rearranged to equation 7:

$$I_t \text{ (mg/ml)} = D_t - (D_0 - I_0)\, e^{\frac{-\text{MTC}\, t}{V}} \quad (7)$$

in which MTC equals the dextran disappearance rate to the interstitium of 0.7 ml/min, V is the volume of distribution of the peritoneal interstitium, I_0 is the initial interstitial concentration, I_t the interstitial concentration after two days, D_0 the initial dextran concentration in the dialysate and D_t the concentration after two days (both 1 g/liter).

After two days of saturation the clearance of dextran ($C_{dextran}$) during a four hour dwell can be calculated as follows:

$$C_{dextran} \text{ (ml/min)} = \frac{\Delta I_t V}{D_0 t} \quad (8)$$

in which ΔI_t is the increase in interstitial dextran concentration from 48 till 52 hours, and t is four hours. The effect of two days of saturation on the dextran concentration within the peritoneal interstitium and on the clearance of dextran from the peritoneal cavity, using different peritoneal interstitial volumes and assuming a lymph flow of 0.3 ml/min, is given in Table 1. In the calculations above, the transport of dextran from blood to interstitium is neglected. The power of our study to detect a difference of 0.45 ml was 85% [33].

Results

No differences were found in the calculated DAR between day 1 and day 3 (1.11 ± 0.56 vs. 0.97 ± 0.41 ml/min, P = 0.23). The data of the individual patients are shown in Figure 1. In the patient studied three times the DAR was 0.32 ml/min on day 1, 0.62 on day 3 and 0.42 on day 5. Probably due to the coefficient of variation of the calculation of DAR, and perhaps also to the intrapatient variation, the values in the two tests were not

Table 2. Peritoneal fluid kinetics obtained before (day 1) and after (day 3) intravenous administration of dextran 70

	Day 1	Day 3
	ml	
Residual volume$_0$	237 ± 64	256 ± 66
Instilled volume	1751 ± 97	1571 ± 97
$TCUF_1$	6.8 ± 1.8	9.4 ± 2.6
$TCUF_{240}$	347 ± 71	257 ± 66
LA_{240}	265 ± 48	232 ± 35
Drained volume	1779 ± 148	1711 ± 105
Residual volume$_{240}$	251 ± 30	314 ± 72

Abbreviations are: TCUF, transcapillary ultrafiltration; LA, lymphatic absorption (calculated as dextran absorption). The subscripts represent the dwell time (minutes). Mean values ± SEM are given. No significant differences were found between the two days.

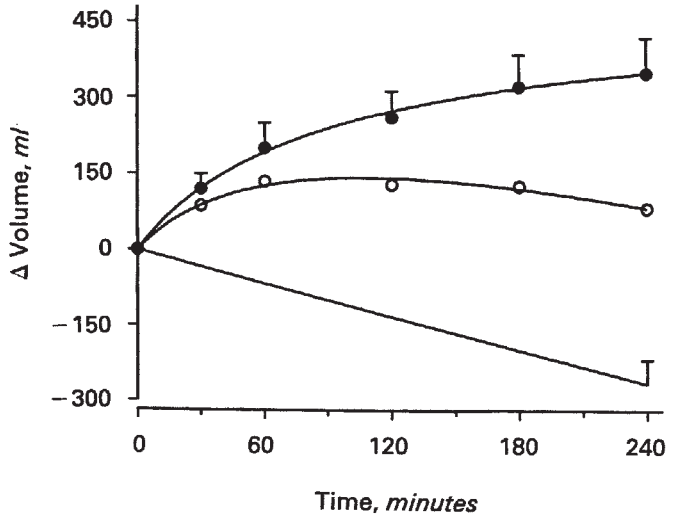

Fig. 2. *The time course of transcapillary ultrafiltration (TCUF) (●), changes in intraperitoneal volume (ΔIPV) (○) and lymphatic absorption (straight line, calculated as dextran absorption) during the first study in eight patients.* The calculated data points and SEM are given. The lines of TCUF and ΔIPV are drawn using the Lineweaver-Burke plot.

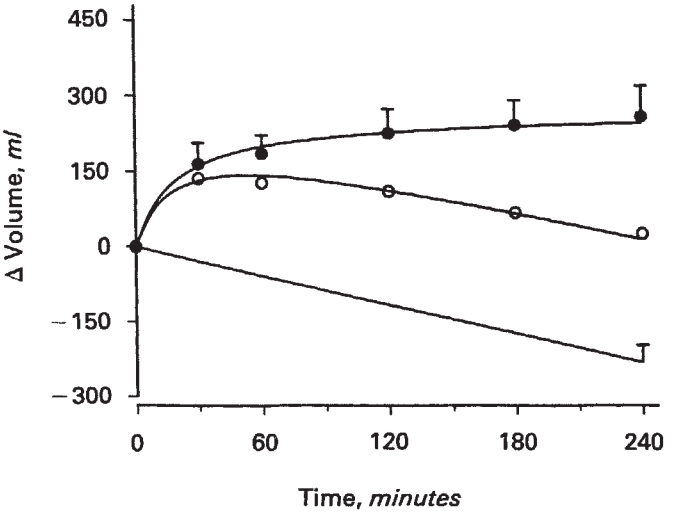

Fig. 3. *The time course of transcapillary ultrafiltration (TCUF) (●), changes in intraperitoneal volume (ΔIPV) (○) and lymphatic absorption (straight line, calculated as dextran absorption) during the second study in eight patients.* The calculated data points and SEM are given. The lines of TCUF and ΔIPV are drawn using the Lineweaver-Burke plot.

Table 3. Peritoneal solute transport characteristics obtained before (day 1) and after (day 3) intravenous administration of dextran 70

	Day 1	Day 3
	ml/min	
Urea	25.4 ± 7.0	24.2 ± 5.6
Lactate	17.1 ± 7.9	16.1 ± 5.8
Creatinine	12.1 ± 4.8	11.6 ± 3.6
Glucose	10.2 ± 3.7	10.3 ± 3.0
Inulin	4.4 ± 1.5	4.6 ± 1.8
Albumin	0.118 ± 0.078	0.103 ± 0.064
IgG	0.068 ± 0.056	0.060 ± 0.036

Mean values ± SEM are given. Values for urea, lactate, creatinine, glucose and inulin are calculated as mass transfer area coefficients, for albumin and IgG as clearances. No significant differences were found between the two days.

always identical. However, values below 0.3 ml/min were never observed. DAR calculated without correction for transcapillary transport was also not different between study 1 and study 2 (1.16 ± 0.56 vs. 0.94 ± 0.41 ml/min, $P = 0.11$). No differences were found between the two studies in TCUF and ΔIPV at the various time points that dialysate was sampled during the test. The volume parameters in the two studies measured at the start and the end of the test and the $TCUF_1$ are given in Table 2. The time course of TCUF, ΔIPV and lymphatic absorption rate (calculated as DAR) in the two studies is shown in Figures 2 and 3. A linear relationship was found between TCUF and ΔIPV ($r = 0.792$, $P = 0.00025$). No relation was found between lymphatic absorption rate (calculated as DAR) and ΔIPV. No differences were found in the peritoneal solute transport characteristics between the two studies (Table 3).

The dextran concentrations in serum at the start of the test on day 3 were 1.3 ± 0.5 g/liter and the end of the test 1.3 ± 0.4 g/liter. Mean dextran concentrations in serum on day 3 ranged from 648 to 1956 mg/liter, with serum concentrations higher than 1 g/liter in six of the eight patients. In the patient studied also on day 5, the dextran concentrations on day 3 and day 5 were 795 and 298 mg/liter.

The residual volume is crucial in calculating the amount of dextran lost during the tests. Therefore we also calculated the residual volume using the dextran data and compared these with the residual volume obtained with the use of inulin. No differences were found between the residual volume calculated with the use of inulin or dextran before the test of day 1 (237 ± 180 vs. 217 ± 166 ml). A good correlation was found between the residual volume calculated with the use of inulin or dextran before the test of day 1 ($r = 0.988$, $P < 0.00001$). No significant correlation was found between the residual volume measured with inulin before and after the test.

As had been found in rats, study 3 confirmed that the

disappearance of dextran in humans was also linear in time ($y = -0.04x + 98$, $P < 0.0001$).

Discussion

In this study no significant differences were found in the disappearance of intraperitoneally administered dextran 70 in a non-steady state condition compared to a steady state. The study is an attempt to demonstrate that lymphatic absorption is represented by the convective leak of macromolecules from the peritoneal cavity. If lymphatic absorption is overestimated by local accumulation, a large difference should have been found between the two tests. Therefore, this study supports the concept that lymphatic absorption can be determined as the disappearance rate from the peritoneal cavity of macromolecular substances. The order of magnitude of lymphatic absorption, calculated by the disappearance of dextran, is in the same order of magnitude as has been found using other markers [3, 15] and as has been found by the comparison of intravenous and intraperitoneally administered inulin [34].

The assumption that lymphatic absorption is represented by the disappearance of macromolecules not only includes the uptake in the subdiaphragmatic lymphatics, but also uptake in the lymphatic system in all other tissues surrounding the peritoneal cavity. This assumption is only valid when the macromolecules enter all lymphatics in the same concentration as present in the dialysate without size restriction. The presence of ^{125}I-human serum albumin in the abdominal wall as has been demonstrated in rats by Flessner et al [19, 20], does not invalidate the method of calculating lymphatic absorption according to Nolph et al [2], provided that the macromolecules in the abdominal wall eventually enter the circulation by the lymphatics. Mactier et al using India ink could not confirm the results of Flessner et al [35].

However, overestimation of lymphatic absorption could occur under the following conditions. Firstly the marker could accumulate in the tissues without entering the circulation. Secondly the marker could accumulate in the tissues in a much higher concentration than present in the dialysate and thereafter enter the circulation by diffusion or uptake by the lymphatic system, while fluid is simultaneously absorbed by the peritoneal capillaries.

When the first possibility holds true, tissue saturation can be expected in a steady state after continuous intraperitoneal administration and a lower disappearance of dextran should have been found. The second possibility has been suggested by Flessner et al [12, 20]. However, in their studies in rats they did not observe tissue over dialysate concentration ratios higher than 1 [20]. The clearances of different fractions of dextran 70 with Einstein-Stokes radii ranging from 35.5 Å to 94.8 Å from blood to dialysate, taken from the literature, are 0.02 to 0.1 ml/min [5]. Therefore very high tissue concentrations of at least ten- to one hundred-fold the dialysate concentration must be present before backdiffusion through the capillary walls can explain the removal of macromolecules from intraperitoneal tissues. In this situation, diffusion from the interstitium back to the dialysate compartment should also occur, giving a lower disappearance of the marker in the second test on day 3.

It is not possible to exclude that a separate uptake of macromolecules by the interstitial lymphatics is present combined with fluid uptake in the peritoneal capillaries by the plasma oncotic pressure, when this occurs in the same ratio as present in the dialysate. Mactier and Khanna have summarized the data in the literature and concluded that in a short dwell during CAPD transperitoneal transport of water from dialysate to blood is not very likely, as a crystalloid osmotic gradient then is still present at the end of the dwell [36]. It implies that during this period the disappearance of macromolecules will accurately describe the fluid disappearance from the peritoneal cavity. When more fluid would be resorbed locally than macromolecules by the lymphatics, again a higher tissue concentration should be present, leading to diffusion to the peritoneal cavity and a lower disappearance of macromolecules during the second test.

It could be argued that the time between the two tests was not long enough for complete tissue saturation. However, this is not very likely in view of the estimated volume of distribution of the interstitial space of the peritoneal membrane. In an earlier study in which dextran 70 was given intravenously, we could demonstrate the presence of a third compartment, possibly the interstitial space of the peritoneum [5]. This compartment was saturated after a period of 38 to 46 hours. In the present study saturation took place for 48 hours both from the circulation and the peritoneal cavity. In one patient DAR remained the same even after five days of intraperitoneal administration of dextran.

Theoretically, part of the disappearance from the peritoneal cavity of dextran could also be explained by either adhesion to the dialysate bag, or uptake in macrophages within the peritoneal membrane. The dialysate samples before inflow of the test bag were taken after the injected dextran was thoroughly mixed and the bag was heated. Therefore adhesion to the bag will not have influenced our results. Dextran 40, although structurally altered, has been found in macrophages in various tissues and organs, when high dosages of dextran were given repeatedly [37]. However, the same disappearance rate of dextran 70 was observed when dextran was given intraperitoneally in a concentration ten times as high as in this study [16]. As the capacity of macrophages in the peritoneal tissues for uptake of dextran must be limited, a lower disappearance rate should have been found in that study. It is also not plausible that macrophages are capable of removing all other macromolecules, that have been used for volume measurements, in the same percentage of the total dose administered. Therefore, it is unlikely that uptake of dextran in peritoneal macrophages is important for the disappearance of intraperitoneal macromolecules. Disappearance from the circulation of macromolecules, caused by uptake in liver, spleen and lymph nodes, has been found in dogs using ^{14}C-dextran [38], and in rats using ^{125}I-polyvinylpyrrolidin [39]. Dextrans are slowly oxidized [38], at a rate of about 70 to 90 mg/kg/day [40]. This could probably explain the rather low serum concentration of dextran in the patient examined on day 5. Removal of macromolecules from the circulation to the reticuloendothelial cells could also explain the low recovery of the total amount administered intraperitoneally.

Net ultrafiltration is dependent of both TCUF and lymphatic absorption rate. However, ΔIPV was correlated only with TCUF. This implies that the lymphatic absorption rate is not the main determinant of net ultrafiltration during a short dwell, as has also been suggested by others [41].

A good correlation was found between the residual volume measurements with either inulin or dextran, indicating that

inulin can be used to measure residual volume accurately. Despite standardization of the test procedure, no correlation was found between the residual volume before and after the test. This implies that it is essential to measure them both in order to describe fluid kinetics accurately.

In conclusion, the disappearance of dextran 70 from the peritoneal cavity in CAPD patients was not influenced by trapping within the peritoneal interstitial tissues. Therefore, the disappearance of dextran can be used as an indirect measurement of lymphatic absorption. As the order of magnitude of lymphatic absorption measured with dextran is in the same order of magnitude as has been found with other macromolecular markers in CAPD patients, this probably indicates that fluid from the peritoneal cavity by lymphatic absorption can be measured accurately by the disappearance of an intraperitoneally-administered macromolecular marker.

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